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Evaluation of Sticking Agents Mixed with *Bacillus thuringiensis* for Control of Douglas-Fir Tussock Moth

usda douglas-fir tussock moth • research & development program •

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Forest Service U.S. Department of Agriculture

Metric Conversions

1 centimeter	=	0.4 inch
1 milliliter	=	0.03 fluid ounce
1 liter	=	0.26 gallon
1 gram	=	0.035 ounce
1 hectare	=	2.5 acres

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U.S. DEPARTMENT OF AGRICULTURE

This research was funded by the USDA Expanded Douglas-fir Tussock Moth Research and Development Program. This paper reports the results of research only. Mention of a pesticide does not constitute a recommendation for use by the USDA, nor does it imply registration under FIFRA as amended. Also, mention of a proprietary product does not constitute an endorsement by the USDA.

Evaluation of Sticking Agents Mixed with* Bacillus thuringiensis *for Control of Douglas-fir Tussock Moth

Reference Abstract

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Eighteen sticking agents were evaluated for their ability to prevent loss of insecticidal activity of *Bacillus thuringiensis* formulations after exposure to simulated rain. Spray formulations containing BioFilm, High Tack Fish Glue, and Nacrylic X4260 and X4445, NuFilm 17, Plyac, and X-Link 2873 were the most active after exposure to rain. About 30 percent of the activity of *Bacillus thuringiensis* spray mixtures without stickers was lost after exposure to 2.54 cm of rain.

KEYWORDS: Pesticide preparations, *Bacillus thuringiensis* , insecticides (-forest pests, bioassay.

RESEARCH SUMMARY
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Eighteen sticking agents were evaluated for their ability to prevent loss of insecticidal activity of *Bacillus thuringiensis* formulations after exposure to simulated rain. The *B. thuringiensis* was mixed in three carrier solutions: water, 25 percent molasses, and 25 percent Sutro, and bioassayed against *Orgyia pseudotsugata* (McDunnough). Of the various stickers tested, only Carboset 525 adversely affected *B. thuringiensis* activity. Spray mixtures containing molasses or Sutro lost more activity after exposure to rain than did the water mixtures, and spray formulations containing BioFilm, High Tack Fish Glue, Nacrylic X4260 and X4445, NuFilm 17, Plyac, and X-Link 2873 were the most active after exposure to rain. About 30 percent of the activity of *B. thuringiensis* spray mixtures without stickers was lost after exposure to 2.54 centimeters of rain.

Introduction

The microbial insecticide, *Bacillus thuringiensis*, has most commonly been mixed as an aqueous suspension for application against forest insects. Sticking agents have sometimes been added to the spray mixtures to increase weatherability by reducing losses of activity from washoff by rain. Lovo 192 and Pinolene were tested by Lewis and Connola (1966) in *B. thuringiensis* applications against gypsy moth, *Lymantria dispar* (L.). Spore platings of spray residues indicated that the spores remained viable for twice as long as in applications without sticker. NuFilm, Chevron sticker, and BioFilm have also been added to *B. thuringiensis* spray mixtures used against forest insects (Yendol et al. 1973, Lewis et al. 1974, Stelzer et al. 1975), but no evaluations were made of the weathering capabilities of the spray mixtures. Laboratory studies by Maksymiuk and Neisess (1975) demonstrated that formulations with NuFilm BT increased weatherability by about 20 percent.

Many sticking agents are available today that have not been tested for compatability or weatherability with *B. thuringiensis* spray mixtures. This paper reports on laboratory screening of the weatherability of several new and old sticking agents. Because *B. thuringiensis* has frequently been mixed with 25 percent molasses or Sutro carriers for forest applications, the stickers were evaluated along with carriers to identify combinations that would increase weatherability under laboratory conditions.

Materials and Methods

Eighteen sticking agents were evaluated (table 1). Each was mixed with *B. thuringiensis* in one of three carrier solutions (water, 25 percent molasses, and 25 percent Sutro 970) and then exposed to three levels of rain, 0, 1.27, and 2.54 cm in a completely randomized factorial experimental design. The experiment was conducted with each of three commercial *B. thuringiensis* products, Biotrol 16K, Dipel SC, and Thuricide 32B. The *B. thuringiensis* concentrations were 2.11 X 10^6 international units (IU)/ml (equivalent to 8 X 10^9 IU/gal--a concentration frequently used in forest applications). Liquid sticker concentrations were 1 percent by volume. Polyhall 295

Table 1--Sticking agents and their sources

Sticking agents	Sources
Adsee 775	Witco Chemical Corp.
BioFilm	Colloidal Products Inc.
Carboset 514H	B. F. Goodrich Chemical Co.
Carboset 525	B. F. Goodrich Chemical Co.
Chevron Spray Sticker	Chevron Chemical Co.
Exhalt 800	Kay-Fries Chemical Inc.
Geon 552	B. F. Goodrich Chemical Co.
High Tack Fish Glue	Norland Products Inc.
Hycar 1872X6	B. F. Goodrich Chemical Co.
Nacrylic X4260	National Starch and Chemical Corp.
Nacrylic X4401	National Starch and Chemical Corp.
Nacrylic X4445	National Starch and Chemical Corp.
NuFilm 17	Miller Chemical
Polyhall 295	Stein, Hall and Co., Inc.
Plyac	Allied Chemical Co.
Texcryl 3662	Poly-Acryls, RA Chemical Corp.
Ultra	Sanico
X-Link 2873	National Starch and Chemical Corp.

and Carboset 525, both solids, were mixed at the rate of 0.125 g/100 ml and 1.0 g/100 ml, respectively. The lower rate was used with Polyhall 295 because of thickening properties of this compound.

The same mixing order and procedure was used with all stickers and treatment combinations. The sticker was added to the amount of water needed to make 100 ml of final mix. This mix was agitated until the sticker had either dissolved or become completely suspended. Carboset 525 required an alkaline solution for solubility; 0.5 ml of ammonium hydroxide was added to the water to create a pH range of 7.5-7.7. Molasses or Sutro was then added at the rate of 25 ml/100 ml of total mix and agitated. Finally, the *B. thuringiensis* product was added and agitated again. Treatment combination mixtures that formed water-insoluble precipitates were not evaluated further.

The effectiveness of the sticking agents was evaluated as follows: 0.2 ml of each spray formulation was sprayed onto 10-cm-long cuttings of new growth Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, using an S.T. 4 Laboratory Spray Tower (Burkard Manufacturing Co., Richmans Worth, England). The volume of deposit on the cuttings was determined to be 11.72 (SD = 3.80) μl/g of foliage, which compares to deposits resulting from a 9.35 l/ha application rate in the field (Stelzer et al. 1977). The spray residues were allowed to dry overnight, in total darkness, at room temperature (20-22°C). One prerain sample, at least 2.0 cm long, was cut from each treated cutting and placed in a 50- by 9-mm petri dish with a tight lid. The remainder of the treated cutting was then exposed to simulated rain.

Rain was simulated, with tapwater, using Monarch misting nozzles (6.4-F100C) positioned 1.3 m above the foliage sample. Rain rates were about 2.54 cm/h. After fir cuttings were exposed to 1.27 or 2.54 cm of rain, samples were cut and placed in petri dishes the same as the prerain samples. Five 3-day-old Douglas-fir tussock moth larvae, *Orgyia pseudotsugata* (McD.), from our laboratory colony were placed on the treated samples in the petri dishes. Larvae were also placed on untreated foliage, which was exposed to rain the same as the treated foliage. Five replicates (five larvae/petri dish per replicate) were tested with each treatment at each rain exposure.

Mortality was recorded after the larvae were held at 25.5 ± 1.0°C (78°F) and 40 ± 5 percent relative humidity for 96 h. The percentage of original activity of the *B. thuringiensis* (OAR) remaining after exposure to rain was based upon mortality of larvae that fed on prerain samples. Larval mortality and OAR were used to measure weatherability of the treatments.

Mortality and OAR data were subjected to analysis of variance. Treatment combinations that formed water-insoluble precipitates upon mixing were treated as missing data. Treatment means were compared using Tukey's w-procedure at the 0.01 level. Data for the three commercial products were analyzed separately.

Results and Discussion

Weatherablity of the various spray formulations varied considerably under the test conditions (tables 2-4). Because many of the sticking agents were acrylic resins or copolymers, they were chemically reactive and formed insoluble precipitates easily (designated by P, tables 2-4). The mixing procedure outlined earlier was designed to dilute the reactive agents as much as possible before combining them. Because even the finest precipitate can plug or reduce the flow in spray system, all mixtures were examined carefully for water-insoluble films.

In a screening experiment of this size, the large number of degrees of freedom in each analysis may result in statistically significant treatment differences that may not be of practical significance, considering that only five

Table 2--Weatherability of *Bacillus thuringiensis* (Dipel SC) mixed in 3 aqueous solutions with various sticking agents, measured at 96 hours by percent mortality and percent original activity remaining (OAR) after exposure to simulated rain[1/]

Sticking agent	Water					25% molasses					25% Sutro				
	Avg. mortality			Avg. OAR		Avg. mortality			Avg. OAR		Avg. mortality			Avg. OAR	
	Rain (cm)			Rain (cm)		Rain (cm)			Rain (cm)		Rain (cm)			Rain (cm)	
	0	1.27	2.54	1.27	2.54	0	1.27	2.54	1.27	2.54	0	1.27	2.54	1.27	2.54
	Percent														
None	74	53	56	78	80	94	58	56	65	64	96	92	84	97	88
Adsee 775	80	76	76	98	97	96	88	88	93	93	96	76	60	79	62
BioFilm	68	44	60	72	92	100	12	12	12	12	100	64	24	64	24
Carboset 514H	P	P	P	--	--	P	P	P	--	--	P	P	P	--	--
Carboset 525	P	P	P	--	--	P	P	P	--	--	60	60	60	111	115
Chevron Sticker	84	100	80	123	98	100	92	96	92	96	84	84	68	117	95
Exhalt 800	76	92	96	126	132	100	76	76	76	76	100	32	20	32	20
Geon 552	P	P	P	--	--	92	68	56	74	61	96	100	68	105	72
High Tack	92	84	92	97	100	96	88	72	93	76	96	64	48	67	51
Hycar 1872X6	P	P	P	--	--	92	60	60	66	68	P	P	P	--	--
Nacrylic X4260	P	P	P	--	--	92	80	68	89	73	P	P	P	--	--
Nacrylic X4401	96	88	92	93	96	96	92	72	97	76	100	52	68	52	68
Nacrylic X4445	84	84	60	132	92	96	92	52	97	55	P	P	P	--	--
NuFilm 17	84	84	96	102	116	92	88	92	96	101	92	76	68	82	74
Polyhall 295	P	P	P	--	--	96	92	24	97	26	P	P	P	--	--
Plyac	96	100	100	105	105	100	84	92	84	92	88	84	80	95	94
Texcryl 3662	88	84	68	98	82	100	92	96	92	96	96	68	80	71	85
Ultra	84	76	76	94	91	80	48	40	73	67	96	52	76	55	80
X-Link 2873	68	96	80	198	147	96	88	96	91	100	92	44	20	48	22
Average	80.6	74.9	73.8	101.9	97.2	94.9	73.7	65.7	79.1	71.1	92.3	67.7	58.8	76.8	67.9

[1/] P = a precipitate formed after mixing.

Table 3--Weatherability of *Bacillus thuringiensis* (Thuricide 32B) mixed in 3 aqueous solutions with various sticking agents, measured at 96 hours by percent mortality and percent original activity remaining (OAR) after exposure to simulated rain[1/]

Sticking agent	Water					25% molasses					25% Sutro				
	Avg. mortality			Avg. OAR		Avg. mortality			Avg. OAR		Avg. mortality			Avg. OAR	
	Rain (cm)			Rain (cm)		Rain (cm)			Rain (cm)		Rain (cm)			Rain (cm)	
	0	1.27	2.54	1.27	2.54	0	1.27	2.54	1.27	2.54	0	1.27	2.54	1.27	2.54
	Percent														
None	77	66	45	92	59	96	57	52	61	54	86	60	54	71	57
Adsee 775	88	60	56	66	74	100	76	32	76	32	76	44	20	59	20
BioFilm	88	72	84	83	96	100	84	60	84	60	92	92	80	102	89
Carboset 514H	72	52	44	65	68	96	64	76	69	79	92	47	44	54	47
Carboset 525	100	100	100	100	100	P	P	P	--	--	96	88	64	93	65
Chevron Sticker	80	48	52	61	66	100	64	48	64	48	92	88	72	97	79
Exhalt 800	80	76	52	96	69	96	88	48	91	50	96	76	40	81	42
Geon 552	88	80	48	93	54	96	40	72	40	75	96	72	64	75	67
High Tack	84	80	76	90	102	100	84	80	84	80	92	80	68	90	75
Hycar 1872X6	88	60	55	74	66	92	76	76	84	84	100	56	48	56	48
Nacrylic X4260	92	100	80	109	86	100	100	84	100	84	96	92	72	95	75
Nacrylic X4401	96	88	92	93	95	92	20	16	25	19	96	44	20	44	20
Nacrylic X4445	96	100	96	105	101	92	88	76	96	83	96	96	52	101	53
NuFilm 17	84	59	56	73	73	100	76	48	76	48	96	76	64	79	67
Polyhall 295	100	92	84	92	84	100	96	84	96	84	96	64	52	66	55
Plyac	96	100	76	105	80	100	96	96	96	96	84	72	48	89	51
Texcryl 3662	84	80	76	99	95	P	P	P	--	--	80	44	16	57	18
Ultra	76	92	48	123	63	92	64	44	72	49	100	64	64	64	64
X-Link 2873	88	96	96	114	114	100	80	60	80	60	100	52	49	52	49
Average	85.0	76.3	64.1	91.4	76.7	97.0	72.0	60.8	74.5	62.7	92.4	68.4	52.3	74.9	54.9

[1/] P = a precipitate formed after mixing.

Table 4--Weatherability of *Bacillus thuringiensis* (Biotrol 16K) mixed in 3 aqueous solutions with various sticking agents, measured at 96 hours by percent mortality and percent original activity remaining (OAR) after exposure to simulated rain[1]

Sticking agent	Water					25% molasses					25% Sutro				
	Avg. mortality			Avg. OAR		Avg. mortality			Avg. OAR		Avg. mortality			Avg. OAR	
	Rain (cm)			Rain (cm)		Rain (cm)			Rain (cm)		Rain (cm)			Rain (cm)	
	0	1.27	2.54	1.27	2.54	0	1.27	2.54	1.27	2.54	0	1.27	2.54	1.27	2.54
	Percent														
None	89	94	70	106	80	96	74	84	77	88	92	92	72	101	80
Adsee 775	96	84	60	88	63	96	48	28	49	28	84	44	20	58	25
BioFilm	100	100	100	100	100	100	100	96	100	96	100	92	68	92	68
Carboset 514H	100	100	88	100	88	96	80	84	84	88	100	88	76	88	76
Carboset 525	P	P	P	--	--	100	72	68	72	68	100	84	100	84	100
Chevron Sticker	84	80	84	94	104	100	92	84	92	84	96	44	48	44	48
Exhalt 800	84	88	92	108	114	96	100	88	105	93	100	68	56	68	56
Geon 552	100	100	96	100	96	P	P	P	--	--	96	88	100	92	105
High Tack	64	56	52	107	100	92	52	32	59	33	92	24	44	29	47
Hycar 1872X6	92	100	92	109	100	96	84	72	88	75	100	100	96	100	96
Nacrylic X4260	100	96	100	96	100	100	88	56	88	56	100	100	88	100	88
Nacrylic X4401	100	100	96	100	96	100	60	72	60	72	100	92	84	92	84
Nacrylic X4445	100	100	100	100	100	100	72	44	72	44	100	84	92	84	92
NuFilm 17	100	96	100	96	100	100	88	88	88	88	92	96	100	105	110
Plyhall 295	92	92	84	101	92	96	44	24	47	25	100	100	92	100	92
Plyac	96	88	92	92	96	100	96	92	96	92	92	84	92	93	101
Texcryl 3662	100	100	64	100	64	P	P	P	--	--	P	P	P	--	--
Ultra	80	76	60	108	67	100	60	52	60	52	84	80	60	98	77
X-Link 2873	88	80	68	87	70	100	92	96	92	96	100	96	92	96	92
Average	92.0	91.1	80.9	100.8	88.8	93.7	74.5	66.3	78.2	70.4	96.0	80.9	76.7	84.6	79.8

[1]P = a precipitate formed after mixing.

larvae were used in each replicate. More importance was placed on consistent weatherability of stickers and carriers than on individual sticker X carrier differences, to identify potential *B. thuringiensis* spray mixtures that can be studied in more detailed laboratory and field experiments.

Analysis of variance of the mortality and OAR data revealed significant differences among stickers, rain levels, and sticker X carrier interaction for all three *B. thuringiensis* products. The sticker X rain interaction was significant only for the OAR data. The significant sticker X carrier interaction indicated that one or more stickers differed in their response to the three carriers. The general trend of high mortality with no rain (prerain) to lower mortality with increased rain is indicative of the nonsignificant sticker X rain interaction. The significant sticker X rain interaction for the OAR data results from the treatments that exhibited increased OAR values with increased rain.

The 18 sticking agents were mostly compatible with the *B. thuringiensis* products and did not adversely effect *B. thuringiensis* activity. Carboset 525, mixed with Dipel SC, was the only sticker that had prerain mortalities that were significantly lower than the no-sticker treatment, when tested by Tukey's test. Tukey's procedure also showed that several of the stickers significantly increased weatherablity contrasted against no-sticker treatments when the effects were averaged for all carriers. Many other stickers increased the weatherability of one or more of the *B. thuringiensis* carrier mixtures but did not consistently increase the weathering with all carriers. Least significant ranges between means calculated by Tukey's-w procedure were ≤11.9 for mortality data and ≤16.4 for the OAR data. For the Dipel mixtures (table 2), Adsee, Chevron sticker, fish glue, Nacrylic 4401, NuFilm 17, Plyac, and Texcryl 3662 had significantly higher mortalities after 2.54 cm of rain than did the no-sticker treatment when all carriers were averaged.

Of those stickers, only Chevron, NuFilm 17, and Plyac had significantly higher average OAR values than the no-stickers. When the Thuricide 32B mixtures (table 3) were averaged for all carriers, mortality and OAR values for mixtures containing BioFilm, Carboset 525, fish glue, Nacrylic X4260 and X4445, Plyac, Polyhall 295, and X-Link 2873 were significantly higher than the no-sticker mixtures. For Biotrol 16K (table 4), the addition of BioFilm, Geon 552, Hycar 1872X6, NuFilm 17, Plyac, and X-Link 2873 significantly increased mortality after 2.54 cm of rain. Only Geon 552, NuFilm 17, and Plyac showed corresponding significant increases in OAR, however.

Significant differences between carriers were also found for all three *B. thuringiensis* products. Carrier X rain interactions were only significant for the mortality data and not for OAR data. Prerain moralities for the 25 percent molasses (94.94, 97.05, and 93.68 percent for Dipel, Thuricide, and Biotrol, respectively, averaged over all sticker treatments) and Sutro carriers (92.29, 92.4, and 96.0 percent) were significantly higher when tested by Tukey's test than the prerain mortalities for the water carrier (80.61, 85.00, and 92.00 percent). Based on observations of frass production, the degree of feeding was also much lower for the molasses and Sutro mixtures than for the water mixtures. Apparently, the larvae quickly ingested a lethal dose when molasses or Sutro was an additive. After 2.54 cm of rain, however, Tukey's procedure showed that the average percent mortalities of the water carrier mixtures (73.76, 64.12, and 80.91 percent for Dipel, Thuricide, and Biotrol, respectively) were significantly higher than mortalities for the 25 percent molasses (65.70, 60.84, and 66.32 percent) or for the 25 percent Sutro mixtures (58.86, 52.27, and 76.67 percent).

Although molasses and Sutro reduced the weatherability of the *B. thuringiensis* mixtures, the higher prerain mortalities indicate these adjuvants could increase the reliability of aerial application, especially if no rain occurred. Because local rain showers are difficult to predict during spray operations, addition of a sticker to a molasses or Sutro spray mixture would provide the best spray formulation for all conditions. Plyac, Texcryl 3662, X-Link 2873, and Chevron sticker significantly increased the weatherability of Dipel + molasses mixtures. No sticker increased weathering of Dipel + Sutro mixtures. Nacrylic X4260 increased the sticking properties of both Thuricide + molasses and Thuricide + Sutro mixtures. NuFilm 17 and X-Link 2873 were the only stickers that increased the weatherability of both molasses and Sutro + Biotrol mixtures. Many stickers increased the weathering of a specific carrier X *B. thuringiensis* product spray mixture, but did not perform consistently for all carriers.

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